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# Haematological, Biochemical and Clinical Signs Changes Following Experimental Infection of *Streptococcus agalactiae* in Red Hybrid Tilapia (*Oreochromis* sp.)

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## Abstract

**Milud Alsaid, Ali F. Abuseliana, Hassan H. Daud, Noordin M. Mustapha, Siti Khairani Bejo, Yasser M. Abdelhadi, and Ruhil H. Hamdan**. **2014**. **Haematological, Biochemical and Clinical Signs Changes Following Experimental Infection of *Streptococcus agalactiae* in Red Hybrid Tilapia *(Oreochromis* sp.*) Aquacultura Indonesiana, 15 (2) : 86-93.*** Hematological and biochemistry parameters are essential for the evaluation of fish health status; it helps provide essential information to diagnose fish diseases. This experiment was conducted to investigate the affect of *Streptococcus agalactiae* infection on hematological and biochemical parameters in red hybrid tilapia (*Oreochromis* sp.). The fish were inoculated intraperitoneally with 1 $x10^4$ cfu/mL bacteria then blood samples were collected and tested during the experiment. After seven days The red blood cell (RBC) counts decreased from 2.31 x $10^6$ to 1.68 x $10^6$. The hemoglobin (Hb) and hematocrit (PCV) counts decreased to 7.17 g/dL and 19.2% in the infected fish compared to the control group (9.71 g/dL and 28.20%). In contrast, the white blood cell (WBC) counts increased from $4.20x10^3$ in the control fish to $13.2x10^3$. The biochemical results initially showed an increase in glucose levels of the infected fish compared to the control from 53.82 to 101.75 on the $72^{nd}$ hour, respectively. There were no significant differences in the levels of serum total protein (T. prot), albumin (ALB), and aspartate transminase (AST) between the infected and the control fish. The inoculated fish displayed marked clinical signs such as erratic swimming behavior, exophthalmia and lethargy during the experimental. The present study has established that the changes in hematological, biochemical and clinical signs of fish can be used as an early diagnosis of pathological and physiological status in red tilapia culture.

***Keywords**:* Biochemical; Clinical signs; Hematological; *Oreochromis* sp.; *Streptococcus agalactiae*

## Introduction

Red hybrid tilapia (*Oreochromis* sp.) is becoming very important commercial venture as they are well accepted by people, it grow fast and has rapid weight gain. Tilapia most widely cultured in ponds, cages and tanks as well as in pen culture systems (Hamzah *et al.,* 2008). The first introduction of red hybrid tilapia *O. niloticus* to Malaysia was in the mid 1980s. Since their introduction, red tilapia production has increased significantly (Siti-Zahrah *et al.,* 2008).

Currently, tilapia is a commonly aquacultured fish and it is an important seafood source for human consumption. Up to date, still little is known about their normal physiology and responses to disease infection. Hence, continuous improvement in the health assessment methods of tilapia is an essential. However, hematology which is frequently used for diagnostic clinical diseases in veterinary world, the techniques are still limited in aquatic animal health medicine (Chen *et al.,* 2003). One of the most significant emerging diseases in tilapia farming in the Asia-Pacific region and particularly in closed systems is caused by *Streptococcus* sp. bacteria.

Streptococcosis is caused by *Streptococcus agalactiae (S. agalactiae)*, which infects both animals and humans causing meningoencephalitis in fish, mastitis in cows, and neonatal sepsis in humans (Pereira *et al.*, 2010). This bacterium affects most of the marine and fresh water fish, including Gulf killifish (*Fundulus grandis) (*Rasheed and Plumb, 1984), red hybrid tilapia (*O. aureus and O. niloticus)*. Eldar *et al.* (1995), sea bream *Sparus auratus*

Evans *et al.* (2002) and silver pomfret (*Pampus argenteus)* Duremdez *et al.* (2004) Streptococcosis in tilapia is the most considered destructive bacterial disease. It is usually caused by *S. agalactiae* Pasnik *et al.* (2009) leading to an acute or chronic, systemic disease with high morbidity and mortality.

In May 2010, mass mortality in red hybrid tilapia occurred on a farm in Selangor state, Malaysia. In an earlier report the mortality was determined to be associated with

*S. agalactiae (*Abuseliana *et al.*, 2010). The knowledge of hematological, biochemical and tissue changes in fish organs have become an important tool for monitoring outbreaks of bacterial fish diseases in laboratory studies (Filho *et al*., 2009).

There have been only a few studies that have evaluated the changes in blood parameters and clinical signs relation to streptococcal disease of fish. Therefore, the objective of this current study is to determine the changes in various hematological, biochemical parameters and clinical changes of adult red hybrid tilapia injected intraperitoneally with *S. agalactiae* which was originally isolated from naturally infected fish.

## Materials and Methods

### *Fish*

One hundred fifty clinically healthy adult red hybrid tilapia (*O. niloticus*) (mean weight 235 g, mean length 21 cm) were obtained from a commercial tilapia farm and then, maintained in 2 fiberglass tanks (3.0 tons each) at the Aquatic Animal Health Unit, Universiti Putra Malaysia for 2 weeks. During the acclimatization period, the fish were fed twice daily with commercial fish feed at a rate of 2% of their body weight until 2 days prior to experimental infection. The aquaria water was monitored and renewed with 50% fresh water daily. Water quality parameters were measured using YSI 85 device, the environmental conditions were kept constant during the experiment (Temperature: 29±1.2°C; DO: 5.8 ± 1.2 mg/L; pH: 7.2 ± 0.3; ammonia: 0.3±0.1 mg/L). During acclimatization, the fish were observed daily for any clinical signs of diseases; eight tilapia specimens were randomly collected for microbiological examination to confirm that the fish were disease free.

### *Bacterial strain and culture of S. agalactiae*

*Streptococcus agalactiae* was originally isolated from a naturally infected red tilapia. The bacteria were identified as *S. agalactiae* group B using commercial identification kits (Streptococcal grouping Kit, RapID™ STR System and BBL Crystal GP ID kit) and compared with American type culture collection (ATCC) (Manassas, VA) Coryne bacterium striatum (ATCC 6940) as reference strains. A stock cultured in brain heart infusion (BHI) broth then stored at -80ºC with 0.85% (w/v) NaCl and 20% (v/v) glycerol to provide for stable inoculates throughout the experimental period. The inoculums were prepared by subculture the bacteria in BHI broth and incubated at 30ºC for 24 hours in shaker, and then, different concentration of the bacteria prepared for the experiment.

### *Experimental infection*

The fish were divided into five groups. Each group of ten fish was kept in a 120 L glass aquarium. Fish in the first four groups were inoculated intraperitoneally (ip) with 1 mL of $1\times 10^4$ cfu/mL of *S. agalactiae*, while the fish in the $5^{th}$ group were injected with 1mL of physiological saline and designated as the control. The bacterial stock was diluted in a saline solution (0.85%) to reach the concentration of $1x10^4$ cfu/mL by a 10-fold serial dilution (1:10). All inoculated fish were observed daily for any clinical signs, abnormal behavior or mortalities for a period of seven days post-infection.

### *Blood sample collection and analysis*

Blood samples were collected at 0, 1, 3, 5, and 7 days- post infection (DPI). Firstly, the fish were anesthetized with tricaine methanesulfonate (MS-222) at a concentration of 50 mg/L. Approximately, 1.5 mL blood samples were drawn from the each fish via the caudal vertebral vein into a heparinized syringe, with 21G needle and immediately transferred into tubes containing lithium heparin anticoagulant. Leucocytes counts (WBC) and erythrocytes counts (RBC) were determined manually utilizing a Neubauer hemocyter using Natt-Herrick's stain (Natt and Herrick, 1952). The hemoglobin concentration (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were estimated using an automated blood analyzer (Abbott Cell-Dyn 3700, Abbott laboratories, Abbott Park IL, USA). An automatic biochemical analyzer (Hitachi 902, Roche Diagnostics) was used to measure the serum total protein (TP), aspartate aminotransferase (AST), Alanine transaminase (ALT) and glucose (GLUC). To confirm the presence of the inoculated bacteria in the blood, thin blood smears were routinely prepared and stained using May Grunwald-Giemsa-Wright as described by reference (Tavares-Dias and Moraes, 2003).

***Statistical analysis***

The data obtained from the biochemical and hematological tests were statistically analyzed using ANOVA and Duncan multiple range test in order to test the significance among groups with SPSS 11 for windows, wherein P-value 0.05 indicates significance

## Results

The haematological and biochemistry parameters results obtained fromthe experimentwere shown in Table I. During the experiment, no mortality was reported. Significant reductions were observed in the hemoglobin seven days after inoculation with *S. agalactiae*. The mean values of RBC, PCV and Hb fluctuated, but they were significantly lower ($P$<0.05) than those of the controls at 3, 5, and 7 dpi. Compared to fish in the control group, the infected fish showed decreased in MCHCH and MCH mean values at 5 and 7 dpi. The WBC mean values of infected fish had higher significant changes at 5 and 7 dpi, respectively.

Biochemical indices of TP, ALT and AST levels means also fluctuated, and there were significant differences ($P$<0.05) between the infected and control groups. The glucose levels in infected fish initially increased significantly ($P$<0.05) from the control mean values of 53.82±10.83 to 87.84 ± 19.58 (mg/dL) and 101.75 ± 24.85 (mg/dL) at 1 and 3 dpi respectively, whereas at 5 and 7 dpi there were no significant differences with respect to the control. When the blood smears of infected fish were stained using Wright's stain, they showed numerous gram positive cocci freely found within the plasma, some attached to the surface of the erythrocytes (Figure 1), and also inside monocytes (Figure 2).

Table 1. Mean values and standard deviations of haematological and biochemical parameters for adult red hybrid tilapia (*Oreochromis* sp.) experimentally inoculated with *S. agalactiae*

| Parameters | Control | 1 DPI | 3 DPI | 5 DPI | 7 DPI |
|---|---|---|---|---|---|
| RBC(x$10^6$/µL) | 2.30±0.17$^a$ | 1.90±0.24$^b$ | 1.73±0.20$^b$ | 1.15±0.36$^c$ | 1.57±0,40$^b$ |
| WBC(x$10^3$/µL) | 4.20±1.8$^a$ | 2.59±1.5$^b$ | 4.18±2.1$^a$ | 7.26±3.3$^{ac}$ | 13.2±5.5$^c$ |
| PCV (%) | 26.20±2.6$^a$ | 24.10±2.1$^{ab}$ | 22.1±1.6$^b$ | 20.10±2.9$^{bc}$ | 19.2±1.8$^{bc}$ |
| Hb(g/dL) | 9.71±0.4$^a$ | 9.10±0.8$^a$ | 8.61±1.3$^{ab}$ | 7.39±1.1$^b$ | 7.17±1.4$^b$ |
| MCV(fL) | 130.5±23.7$^a$ | 122.71±15$^a$ | 115.8±10$^a$ | 118.7±11$^a$ | 100.8±13.5$^a$ |
| MCH(pg) | 52.7±4.6$^a$ | 49.7±5.3$^a$ | 48.70±3.5$^{ab}$ | 47.1±2.9$^{ab}$ | 42.4±4.2$^b$ |
| MCHC(g/dL) | 43.61±5.8$^a$ | 42.50±1.3$^{ab}$ | 38.10±1.5$^c$ | 36.90±1.4$^c$ | 37.60±2.2$^c$ |
| Total protein(g/dL) | 3.15±0.26$^a$ | 2.71±0.30$^a$ | 2.50±0.20$^b$ | 2.28±0.19$^{bc}$ | 2.34±0.15$^{bc}$ |
| Glucose(mg/dL) | 53.8±10.7$^a$ | 87.8±19.6$^{ab}$ | 101.8±24.9$^b$ | 66.3±32.1$^{ab}$ | 57.8±11.$^{7ab}$ |
| ALT(U/L) | 22±0.35$^a$ | 22±0.90$^a$ | 25±0.30$^b$ | 28 ±0.60$^c$ | 27±0.45$^b$ |
| AST(U/L) | 83±0.54$^a$ | 83±0.61$^a$ | 88±0.43$^b$ | 100±0.72$^c$ | 97±0.35$^d$ |

$^{a, b, c, d}$ Mean (±SEM) with the same letter in each row are not significantly different $P \leq 0.05$.

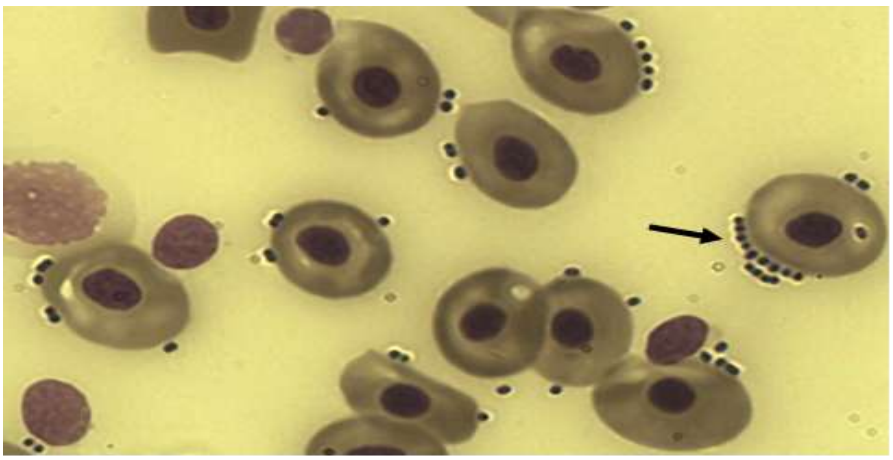

Figure 1. Peripheral blood smear of red hybrid tilapia infected with *S. agalactiae* showing the bacteria cells attached to red blood cells wall (arrow) (Wright's stain, 1000x).

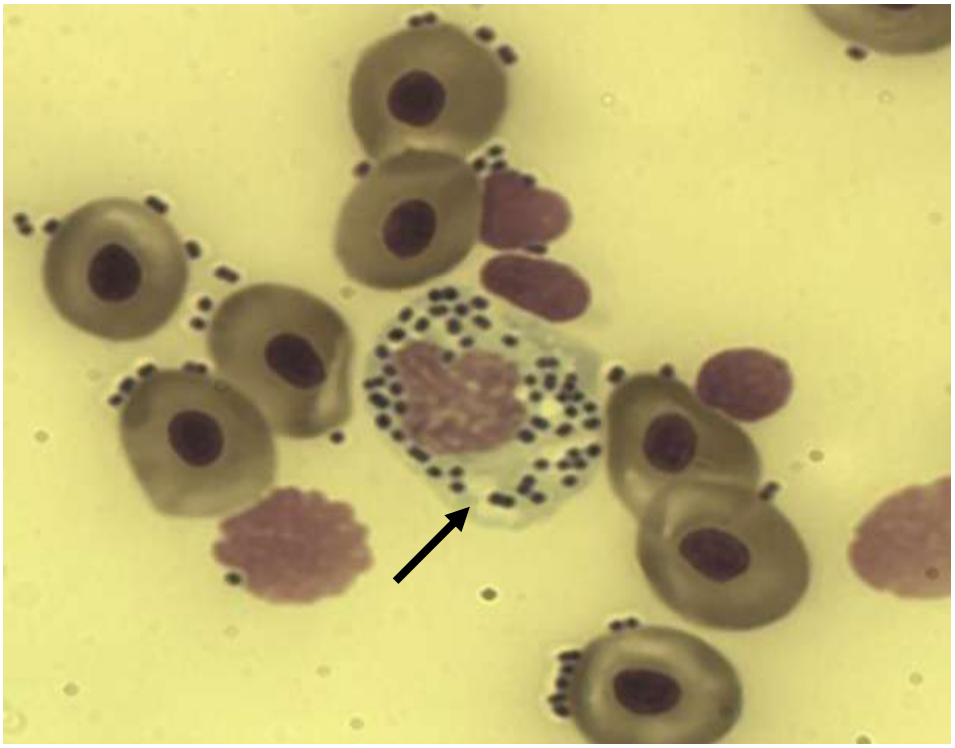

Figure 2. Peripheral blood smear of red hybrid tilapia infected with *S. agalactiae* showing bacteria cells engulfed by monocytes. (arrow) (Wright's stain, 1000x).

There were no significant observations in mortality, clinical and pathological changes in the control group. On the 1st dpi, most of the fish started to show signs of streptococcal disease, such as staying motionless on the aquarium bottom and lethargic. On the 3rd dpi, the infected fish were still lethargic with rapid erratic swimming. One the 5th and 7th dpi fish showed exophthalmia (Figure 1), opaqueness of the eyes and faecal strings (Figure 2).

## Discussion

Haematological, biochemical and clinical signs changes have been studied in many fish species during bacterial infection to identify the variable parameters for determination of healthy and diseased fish. The present study describes details of changes in clinical, haemotological and biochemical parameters induced by *S. agalactiae* infection in red hybrid tilapia.

Hematological and biochemical analysis have been studied in many fish species cultured during bacterial infection to Identify of variable parameters to determine healthy and diseased fish.

Current finding indicated that there was significant decreased in red blood cells, hemoglobin and hematocrit levels ($P$<0.05). A reduction in Hb and RBC concentration of cichlid fish infected with *Aeromonas* and *Streptococcus* were previously reported (Řehulka, 2002 and McNulty *et al.,* 2003). Therefore, we postulated that the decreased in RBC count, PCV and Hb values were due to the destruction of the haemotopoietic organs which were located in the spleen and pronephros. In a previous study, we reported that red hybrid tilapia infected with *S. agalactiae* showed marked liver necrosis and kidney congestion (Abuseliana *et al.,* 2010). Similarly, earlier reports had shown that *Streptococcus* sp. could cause pathologies in haemopoietic organs, particularly in kidney, spleen and liver of fish (Chang and Plumb, 1996; Chen *et al.,* 2004 and Suanyuk *et al.,* 2005). Reference Yu *et al.* (2010) observed that there was a significant reduction in erythrocytes in peripheral blood, hematocrit percentage and hemoglobin rate, which are indicators of anemia. Similar findings were also reported by reference (Rajendiran *et al.,* 2008) in spotted snakehead, *Channa punctatus*, challenged with *A. hydrophila*. Our current finding also indicated that a sharp reduction in these values which could have affected the oxygen and nutrients transportation to tissues, which was similar to findings of previous studies (Harikrishnan *et al.,* 2009 and Martins *et al.,* 2008). These data

support the present finding that the significant decrease in erythrocyte, hematocrit and hemoglobin content is possibly due to hypochromic microcytic anemia caused by the bacteria.

The evaluated haematological indices, MCHC, MCH, and MCV have a particular importance in the diagnosis of anaemia in most animals Coles (1986). Apparently, there were significant decreases ($P$<0.05) in these blood indices (MCH and MCHC) may be related to the decrease in RBC, Hb and PCV due to disturbances that occurred in hematopoietic organs of fish challenged with *S. agalactiae*. Similar to our results, reference Ranzani-Paiva *et al.* (2004) reported that there was a decreased in the RBC indices such as MCH and MCHC in *Oreochromis aureus* infected by *Corynebacterium* sp. Furthermore, reference Haniffa and Mydeen, (2010) demonstrated that catfish (*Silurus asotus*) exhibited a decrease in MCH and MCHC during *A. hydrophila* infection. They suggested that the decrease in erythrocytes indices of fish may be sign of hypochromic microcytic anemia. A significant decrease in the MCHC after *S. agalactiae* infection is probably an indication of RBC swelling and/or a decrease in haemoglobin synthesis. Although no significant differences were observed in MCV level, it decreased slightly during the challenge period. Similar findings were also observed in Nile tilapia (*O. niloticus*) naturally infected with *Flavobacterium columnare* (Sebastião *et al.*, 2011). Leukocytes in blood are the most important cellular components of the fish immune system to defend against bacteria and foreign materials. In our current experiment, the value of the total leucocytes count was significantly higher in the challenged groups at days 5 and 7 pi than in the control group. This is a natural response to the presence of the bacterial pathogen by the induction of the non-specific defense system. As indicated earlier, leucocytosis generally occurred after bacterial infections in fish (Dalmo *et al.*, 1997; Yildiz, 1998 and Caruso *et al.*, 2002).

By light microscopy, after 24 hours, it was observed that the infected fish had several Cocci free within the plasma, adhering to the surface of the erythrocytes and intracellularly within monocytes. In addition, some changes were observed in the morphology of monocytes that showed numerous larger vacuoles as demonstrated in a previous study of *Mycobacterium marinum* by reference (Ranzani-Paiva *et al.*, 2004). The present study was the first attempt to measure ALT, AST and TP activity in *S. aglacatiae* infected tilapia's blood. Determinations of activities of AST and ALT in serum are a good indicator of bacterial infection of the challenged fish (Bin and Xiao-jin, 2010). In the current study, we have found that there were significantly increased in the activities of both AST and ALT in the serum of infected groups on days 5 and 7 dpi as compared to the control. Elevation of plasma ALT and AST following bacterial infection have been reported in Atlantic salmon (*Salmosolar*) (WaagbØ *et al.*, 1988), Nile tilapia (*O. niloticus*) (Chen *et al.*, 2004) and Brook trout (*Salvelinus fontinalis*) (Řehulka and Minařík, 2007).

The elevations in AST and ALT activities throughout the period of infection were suggested to be due to the severe damage of viscera organs such as liver and kidney. This agrees with reference Khalil *et al.* (2011) findings where they had demonstrated that increased in AST and ALT levels in plasma was associated with hematopoietic organs damage in the experimentally infected *Anguilla anguilla* with *Vibrio anguillarum*.

In the current study, the total serum protein level was reduced by *S. agalactiae* infection, which was consistent in *Edwardsiella tarda* infected tilapia as reported by Reference (Benli *et al.*, 2004). Many recent reports had demonstrated that reduction in total protein occurred in a variety of fish species infected with pathogenic bacteria such as *Flavobacterium columnare*, *Aeromonas* hydrophila, *Staphylococcus xylosus* and *Vibrio anguillarum* (Řehulka and Minařík, 2007; Maqsood *et al.* 2009 and Al-Dohail *et al.*, 2011). Moreover, reference Yu *et al.* (2010) reported that a decrease in the TP level had been observed in catfish (*Silurus asotus)*, experimentally infected with *E. tarda*. Furthermore, they suggested that a low TP level in infected fish might be contributed by to the reduced hepatic protein synthesis and renal re-absorption. In the current experiments, the histopathological changes in the liver and kidney of red hybrid tilapia were observed after *S. agalactiae* infection.

From the findings led us to conclude that reduced levels of total protein in the blood of infected fish could be due to dysfunction of renal excretion and or impaired protein synthesis by the liver. It was observed also in the current study that there was an increase in the serum glucose level in the experiementally infected fish. It

elevated significantly ($P<0.05$) on days 1 and 3 pi. These elevations might be related to the stress response and metabolism. Similar increased in blood glucose levels had been reported by (Evans *et al*., 2006) following exposure of Nile tilapia (*O. niloticus)* to unionized ammonia.

In the present study, there were marked signs of streptococcal disease in the experimentally infected red hybrid tilapia. Similar findings were recently reported with Japanese flounder (*Paralichthysolivaceus)* (Dumrongphol *et al.*, 2009), red hybrid tilapia (*Oreochromis* sp.) Alsaid *et al.* (2010), Red porgy (*Pagruspagrus*) El Aamri *et al.* (2010), Sunshine bass (*Morone chrysops* x *M. saxatilis*) Bowker *et al.* (2010). Interestingly, during current study whitish stringy fecal casts were observed among the infected fish. This was likely due to presence of sloughed intestinal wall cells mixed with mucus in the feces. This clinical sign of fecal string was in agreement with recent report by reference (Pasnik *et al.*, 2009).

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## References

**Abuseliana, A., H. Daud, S.A. Aziz, S.K. Bejo, and M. Alsaid.** 2010. *Streptococcus agalactiae* the etiological agent of mass m:ortality in farmed Red Tilapia (*Oreochromis* sp.). *Journal of Animal and Veterinary Advances*, 9 (20): 2640-2646.

**Al-Dohail, M.A., R. Hashim, and M. Aliyu-Paiko.** 2011. Evaluating the use of *Lactobacillus acidophilus* as a biocontrol agent against common pathogenic bacteria and the effects on the haematology parameters and histopathology in African catfish *Clarias gariepinus* juveniles. *Aquaculture Research*, 42 (2): 196-209.

**Alsaid, M., H. Daud, S.K. Bejo, and A. Abuseliana.** 2010. Antimicrobial activities of some culinary spice extracts against *Streptococcusagalactiae* and Its prophylactic uses to prevent Streptococcal infection in red hybrid tilapia (*Oreochromis* sp.). *World Journal of Fish and Marine Sciences*, 2 (6): 532-538.

**Benli, K., A. Cağlan, and H.Y. Yildiz.** 2004. Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*. *Aquaculture Research*, 35(14): 1388-1390.

**Bin, X. and X. Xiao-jin.** 2010. Studies on blood chemistry indices and histopathology of Pseudosciaena crocea artificially challenged with *Vibrio harveyi*. *Journal of Fisheries of China*, 34(4): 618.

**Bowker, J.D., V.E. Ostland, D. Carty, and M.P. Bowman.** 2010. Effectiveness of Aquaflor (50% florfenicol) to control mortality associated with *Streptococcus iniae* in freshwater-reared subadult sunshine bass. *Journal of Aquatic Animal Health*, 22 (4): 254-265.

**Caruso, D., O. Schlumberger, C. Dahm and J.P. Proteau.** 2002. Plasma lysozyme levels in sheatfish *Silurus glanis* (L.) subjected to stress and experimental infection with *Edwardsiella tarda*. *Aquaculture Research*, 33(12): 999-1008.

**Chang, P. and J. Plumb.** 1996. Histopathology of experimental *Streptococcus* sp. infection in tilapia, *Oroochromis niloticus* (L.), and channel catfish, *Ictafurus punctatus* (Ratinesque). *Journal of Fish Diseases*, 19(3): 235-241.

**Chen, C.Y., G.A. Wooster, and P.R. Bowser.** 2004. Comparative blood chemistry and histopathology of tilapia infected with *Vibriovulnificus* or *Streptococcusiniae* or exposed to carbon tetrachloride, gentamicin, or copper sulfate. *Aquaculture*, 239(1): 421-443.

**Chen, C.Y., G.A. Wooster, R.G. Getchell, P.R. Bowser, and Timmons.** 2003. Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. *Aquaculture*, 218 (1–4): 89-102.

**Coles, E.H.** 1986. *Veterinary Clinical Pathology*. Philadelphia: Saunders Company.

**Dalmo, R.A., K. Ingebrigtsen, and J.Bøgwald.** 1997. Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *Journal of Fish Diseases*, 20(4): 241-273.

**Dumrongphol, Y., T. Hirota, H. Kondo, T. Aoki, and I. Hirono**. 2009. Identification of novel genes in Japanese flounder (*Paralichthys olivaceus*) head kidney up-regulated after vaccination with *Streptococcusiniae* formalin-killed cells. *Fish and Shellfish Immunology*, 26(1): 197-200.

**Duremdez, R., A. Al-Marzouk, J.A. Qasem, A. Al-Harbi, and H. Gharabally.** 2004. Isolation of *Streptococcusagalactiae* from cultured silver pomfret, *Pampus argenteus* (Euphrasen), in Kuwait. *Journal of Fish Diseases*, 27(5) : 307-310.

**El Aamri, F., D. Padilla, F. Acosta, M.J. Caballero, J. Roo, J. Bravo, J. Vivas, and F. Real.** 2010. First report of *Streptococcusiniae* in red porgy (*Pagrus pagrus*, L.). *Journal of Fish Diseases*, 33(11): 901-905.

**Eldar, A., Y. Bejerano, A. Livoff, A. Horovitcz, and H. Bercovier.** 1995. Experimental streptococcal meningo-encephalitis in cultured fish.*Veterinary Microbiology*, 43(1): 33-40.

**Evans, J.J., P.H. Klesius, P.M. Gilbert, C.A. Shoemaker, M.A. Al Sarawi, J. Landsberg, R. Duremdez, A. Al Marzouk, and S. Al Zenki.** 2002. Characterization of β-haemolytic Group B *Streptococcusagalactiae* in cultured seabream, *Sparus auratus* L., and wild mullet, *Liza klunzingeri* (Day), in Kuwait. *Journal of Fish Diseases*, 25(9): 505-513.

**Evans, J.J., D.J. Pasnik, G.C. Brill, and P.H. Klesius.** 2006[a]. Un-ionized ammonia exposure in Nile Tilapia: toxicity, stress response, and susceptibility to *Streptococcusagalactiae*. *North American journal of aquaculture*, 68(1): 23-33.

**Filho, C.I., E.E. Muller, L.G. Pretto-Giordano, and A.F. Bracarense.** 2009. Histological findings of experimental *Streptococcusagalactiae* infection in nile tilapia (*Oreochromisniloticus*). *Brazilizn Journal of Veterinary Pathology*, 2(1): 12-15.

**Hamzah, A., N.H. Nguyen, R.W. Ponzoni, N. Kamaruzzaman, and B. Suhba.** 2008. Performance and survival of three red tilapia strains (Oreochromis spp) in pondenvironment in Kedah state, Malaysia. In H. Elghobashy, K. Fitzsimmons, and A.S. Diab, (eds.) *Eighth International Symposium on Tilapia in Aquaculture* (pp. 199-211). Cairo, Egypt: EISTA.

**Haniffa, M.A. and A.K. Mydeen.** 2010. Hematological changes *in Channa striatus* Experimentally Infected by *Aeromonas hydrophila*. *Bioresearch Bulletin*, 4, 246-253.

**Harikrishnan, R., C. Balasundaram, M.C. Kim, J.S. Kim, Y.J. Han, and M.S. Heo.** 2009. Innate immune response and disease resistance in Carassius auratus by triherbal solvent extracts. *Fish & Shellfish Immunology*, 27(3): 508-515.

**Khalil, R.H., R.E. Hana, and B. Nadia.** 2011. Contribution to Vibriosis in cultured eels (*Anguilla Anguill*). *Journal of American Science*, 7 (12): 101-110.

**Maqsood, S., M. Samoon, and P. Singh.** 2009. Immunomodulatory and growth promoting effect of dietary levamisole in *Cyprinus carpio* fingerlings against the challenge of *Aeromonas hydrophila*. *Turkish Journal of Fisheries and Aquatic Sciences*, 9 (1): 111-120.

**Martins, M., J. Mouriño, G. Amaral, F. Vieira, G. Dotta, A. Jatobá, F. Pedrotti, G. Jerônimo, and C. Buglione-Neto.** 2008. Haematological changes in Nile tilapia experimentally infected with *Enterococcus* sp. *Brazilian Journal of Biology*, 68(3): 657-661.

**McNulty, S.T., P.H. Klesius, C.A. Shoemaker, and J.J. Evans.** 2003. Hematological changes in Nile tilapia (*Oreochromisniloticus*) infected with *Streptococcus iniae* by nare inoculation. *Journal of the World Aquaculture Society*, 34 (3): 418-422.

**Pasnik, D.J., J.J. Evans, and P.H. Klesius.** 2009. Fecal Strings Associated with *Streptococcusagalactiae* Infection in Nile Tilapia, *Oreochromisniloticus*. *The Open Veterinary Science Journal*, 3, 6-8..

**Pereira, U.P., G.F. Mian, I.C.M. Oliveira, L.C. Benchetrit, G.M. Costa, and H.C.P. Figueiredo.** 2010. Genotyping of *Streptococcusagalactiae* strains isolated from fish, human and cattle and their virulence potential in Nile tilapia. *Veterinary Microbiology*, 140 (1–2): 186-192.

**Rajendiran, A., E. Natarajan, and P. Subramanian.** 2008. Control of *Aeromonashydrophila* Infection in Spotted Snakehead, *Channapunctatus*, by *Solanumnigrum* L., a Medicinal Plant. *Journal of the World Aquaculture Society*, 39(3): 375-383.

**Ranzani-Paiva, M.J.T., C. M. Ishikawa, A.C. Eiras, and V.R. Silveira.** 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia, *Oreochromisniloticus* (Linnaeus, 1757). *Brazilian Archives of Biology and Technology*, 47(6): 945-953.

**Rasheed, V. and J.A. Plumb.** 1984. Pathogenicity of a non-haemolytic Group B *Streptococcus* sp. in gulf killifish (*Fundulus grandis* Baird and Girard). *Aquaculture*, 37(2): 97-105.

**Řehulka, J.** 2002. *Aeromonas* causes severe skin lesions in rainbow trout (*Oncorhynchus mykiss*): clinical pathology, haematology, and biochemistry. *Acta Veterinaria Brno*, 71(3): 351-360.

**Řehulka, J. and B.Minařík.** 2007. Blood parameters in brook trout Salvelinus fontinalis (Mitchill, 1815), affected by *columnaris* disease. *Aquaculture Research*, 38(11): 1182-1197.

**Sebastião, F., D. Nomura, R. Sakabe, and F. Pilarski.** 2011. Hematology and productive performance of nile tilapia (*Oreochromis niloticus*) naturally infected with *Flavobacterium columnare*. *Brazilian Journal of Microbiology*, 42 (1): 282-289.

**Siti-Zahrah, A., B. Padilah, A. Azila, R. Rimatulhana, and R. Shahidan.** 2008. Multiple streptococcal species infection in cage-cultured red tilapia but showing similar clinical signs. In M.G. Bondad-Reantaso, C.V. Mohan, M. Crumlish., and R.P. Subasinghe (Eds.) *Diseases in Asian Aquaculture VI* (pp. 313-320). Manila, Philippines: Asian Fisheries Society.

**Suanyuk, N., H. Kanghear, R. Khongpradit, and K. Supamattaya.** 2005. *Streptococcus agalactiae* infection in tilapia (*Oreochromisniloticus*). *Songklanakarin Journal of Science and Technology*, 27(1): 307-319.

**Tavares-Dias, M. and F.R. Moraes.** 2003. Características hematológicas da *Tilapia rendalli* Boulenger, 1896 (Osteichthyes: Cichlidae) capturada em "Pesque-Pague" de Franca, São Paulo, Brasil. *Bioscience Journal*, 19: 103-110.

**Waagbø, R., K. Sandnes, S. Espelid, and Ø. Lie.** 1988. Haematological and biochemical analyses of Atlantic salmon, *Salmo solar* L., suffering from coldwater vibriosis ('Hitra disease'). *Journal of Fish Diseases*, 11(5): 417-423.

**Yildiz, H.** 1998. Effects of experimental infection with Pseudomonas fluorescens on different blood parameters in carp (*Cyprinus carpio* L.). *The Israeli Journal of Aquaculture-Bamidgeh*, 50(2): 82-85.

**Yu, J.H., J.J. Han, and S.W. Park.** 2010. Haematological and biochemical alterations in Korean catfish*Silurus asotus*, experimentally infected with *Edwardsiella tarda*. *Aquaculture Research*, 41(2): 295-302.

**Natt, M.P. and C.A. Herrick.** 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poultry Science*, 31(4): 735-738.